March 9, 1948.

Dr. Michael Doudoroff,
Department of Bacteriology,
University of California.

Dear Dr. Doudoroff,

First, I want to thank you for having sent so many of your reprints when I requested them. They have been very helpful.

Since my arrival here, I have been working on the genetic control of the fermentation enzymes of Escherichia coli, K-12. The approach has been to irradiate dense suspensions on EMB plates containing a given sugar, and to pick up fermentation mutants among the survivors by the failure of the colony (or sector thereof) to turn purple, in contrast to the fermenting, non-mutants. With appropriate technique, as many as 1:1000 colonies will be lactose- or maltose- negative mutants, which has made it very easy to accumulate a large collection of diverse mutants. By the use of recombination techniques it has been possible to classify the mutants obtained genetically, and for example, mutations at any one of seven or eight loci will produce a lactose negative (& glucose-or galactose-positive) mutant. This observation suggests either that there is a very complex enzymatic scheme indeed for the diddimilation of lactose, or that there is a more complex genetic control of enzyme formation than has been thought. Although I am inclined to the latter, the point is not settled, and I must look forward to a lot of chemical work to clear it up. I have a few observations on the specificities of some mutants which may, on the one hand, interest you; on the other, I should like to hear whether you have encountered anything similar in your experience.

The wild type (K-12) ferments hexoses, hexitols, lactose, maltose, melibiose and trehalose, but not cellobiose, gentiobiose or sucrose. Intensive attempts to select out sucrose+ mutants have failed.

Although melibiose is attacked, no utilization of raffinose can be detected, nor have I been successful in selecting out raffinose+ mutants. Is this differential between melibiose and raffinose a frequent observation?

2. After irradiation a mutant (W-108) unable to ferment (or utilize for growth under aerobic conditions) glucose was isolated, On subsequent testing it was found not to ferment maltose or lactose, while it attacked galactose, pentoses, and gluconic acid. In the 108 stock, after heavy inoculation into glucose medium, a strain was developed which fermented glucose, but still not lactose or maltose or trehalose. Genetic study showed that this adapted stock still carried the mutant gene of W-108 (Lac_3-) but that another gene (Sl_3) had mutated so as partially to suppress the effects pf the Lac_3 mutation. By a similar procedure, the mutation Sl_1 which leads to Glu+Lac+Mal- pattern, was selected for in W-108. So far, while genetically interesting, none of this is very startling. However, I was surprised to find, from Maltose selections, still a third mutation, Sl_2+ which suppressed part of the effects pf Lac_3-, to give a strain which is glucose-negative, maltose-positive! I suppese that these "suppressor" mutations are merely opening up alternate pathways, possibly a maltose-phosphorylase, but this remains to be shown. Have you found any clearcut indication of a phosphorylation of maltose or of lactose in any material?

3. Another mutation has recurred (W-145), the locus labelled Lac_5 which has a particularly interesting pattern: Lac-Mal-Glu+Gluconic-. The metabolism of gluconic acid in E. coli is something we know very little about. As such, it is probably not an intermediate of glucose utilization becuase glucose-adapted cells must be adapted to gluconic before they will ferment(sic) it. This mutation has recurred three or four times, and it is very unlikely that the pattern is merely a coincidence. Maltobionic and lactobionic acids are not utilized even by wild type coli, so it is not a question of the first step being oxidation of disaccharides to the bionic acid. Unlike W-108, however, I have not been able to select for specific suppressors of the mutation; i.e., whenever an adaptation to one of the sugars is found, it constitutes a reversion to the wild type. Have you any suggestions as to a possible simple meaning of such a pattern?

4. Have you any data on trehalose breakdown? Some maltose - mutants are trehalose+, others are trehalose-. The Sl_3 Lac_3-, maltose+ strain mentioned above is trehalose-.

5. I am very much interested in the behavior on analogues of the sugars. For example, most of the Lac- mutants (L_1, 2, 4, 6, & 7) are specific, and still ferment maltose, glucose, etc. However, Lac_1- utilizes b-methyl galactoside, Lac_2- does not, but an allelic mutation can be selected for which is Me.gal-, lac+, nor do the others. If you should happen to have access to any other analogues of lactose (synthetic galactosides, allo-lactose, a-l-arabinosides) I would appreciate very much the favor of sufficient samples (downwards of 1 g.) to determine the usefulness of having them synthesized in larger amount).

Your discussion of any of these items would be greatly appreciated.

Yours sincerely,

Joshua Lederberg
Assistant Professor of Genetics.